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Time-dependent effect of chlorhexidine surgical prep

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SUMMARY

Despite continued advances in preoperative preventive measures and aseptic technique, surgical site infections remain a problem. The purpose of this study was to evaluate the time-dependent effectiveness of chlorhexidine, a common surgical preparation solution, at various concentrations. Agar plates containing a Mueller–Hinton medium were inoculated with *Staphylococcus aureus* (lux) bacteria. The bacteria are genetically engineered to emit photons, allowing for quantification with a photon-counting camera system. Standardized amounts of aqueous chlorhexidine at three different concentrations (group 1:4%; group 2:2%; group 3:0.4%) were applied to the agar plates and comparisons in bacterial reduction were made. After 2 min of contact time, groups 1 and 2 had similar reductions in bacterial load with 30% bacterial load remaining in each group ($P = 0.512$), whereas group 3 had a significantly higher bacterial load (33%) when compared to both groups 1 and 2 (1 vs 3, $P < 0.0001$; 2 vs 3, $P = 0.0002$). The bacterial load in all three groups continued to decrease out to the final time point (1 h) with group 1 having the least amount of bacterial load remaining, 9% ($P < 0.0001$) and group 3 with the highest bacterial load remaining, 19% ($P < 0.0001$). This study demonstrates two key results: first, dilution of chlorhexidine correlates directly with its bactericidal activity; second, its effectiveness is directly related to its contact time. Based on the results of this study, the authors recommend using 4% chlorhexidine for surgical site preparation and allowing a minimum of 2 min of contact time prior to making the skin incision.

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Introduction

Despite continued advances in preoperative preventive measures and aseptic technique, up to half a million patients in the USA develop a surgical site infection (SSI) annually.[1] Many different variables may influence the risk for SSI. Patient-related factors include age, nutrition status, diabetes, smoking and obesity, among others. Operative factors must also be taken into account, such as the preoperative skin prep, antimicrobial prophylaxis, and aseptic surgical technique. Although surgeons may attempt to optimize patient-related factors, little may be done in the preoperative period, especially in emergent or urgent situations. However, the surgeon has a significant influence on the operative factors and every effort must be made by the surgical team to minimize the risk for SSI.

In an effort to reduce the likelihood of SSI, preoperative skin antisepsis is performed immediately prior to the surgical procedure. There are many different types of preoperative skin preparation solutions such as povidine–iodine 7% scrub/10% paint (Scrub Care®, Cardinal Health, Dublin, OH, USA) and chlorhexidine gluconate 4% (Hibiclens®, Mölnlycke Health Care, Anderson, SC, USA). Currently, the Center for Disease Control and Prevention has not mandated specific guidelines regarding the type of surgical prep solution for preoperative skin antisepsis. Despite this, recent evidence suggests the superiority of an aqueous chlorhexidine scrub followed by isopropyl alcohol paint or the use of ChloraPrep® (Cardinal Health), which is commercially available as single application of 2% chlorhexidine gluconate and 70% isopropyl alcohol.[1–3]

Chlorhexidine, a common surgical preparation solution, is often used in various concentrations; most commonly 4%, with few data describing how changes in the concentration affect its bactericidal activity.[4,5] Whereas manufacturers' guidelines typically recommend 2 min of contact time prior to removal or surgical incision, there are no published data demonstrating the

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effect of chlorhexidine over time.[6] Due to the busy pace in the operating room, the skin incision may be made prior to 2 min of surgical prep contact time. In an effort to minimize the risk of an SSI related to a surgeon-controlled variable, the preoperative skin prep, we sought to evaluate the time-dependent effectiveness of aqueous chlorhexidine at various concentrations.

Methods

Mueller–Hinton plates were inoculated with *Staphylococcus aureus* (lux) (Xenogen 29; Caliper Life Science, Hopkington, MA, USA). Starting at the top of the plate, the bacteria were spread back and forth covering the entire plate. The plate was then rotated 60 degrees and the process repeated. This process was repeated a third time. The plates were then allowed to replicate in an incubator over a 24 h period. These bacteria are genetically engineered to be luminescent by random chromosomal insertion of the luciferase–luciferin construct.

Each plate was then placed within a dark-box, the IVIS100 imaging system (Xenogen Corporation, Alameda, CA, USA), to obtain baseline luminescent data. This system uses an optical charge-couple device camera to count photon emissions. Imaging software (LivingImage V. 2.12; Xenogen Corporation, Alameda, CA, USA; and IGOR v.4.02A, WaveMetrics, Lake Oswego, OR, USA) was used to superimpose the photon count on to a grey-scale background image yielding the location and photon intensity, which correlates with bacterial quantity.

Three different aqueous chlorhexidine gluconate solutions were tested (group 1: 4%, group 2: 2%, and group 3: 0.4%). Chlorhexidine gluconate 4% was used as a base and serial dilutions were made with sterile water to obtain the additional concentrations. Following baseline imaging, 0.2 mL drops of each concentration of chlorhexidine were placed on to the Mueller–Hinton plates containing the bioluminescent *S. aureus*. The plates were immediately placed back within the dark-box, at which point sequential imaging was performed (Figure 1). Imaging was performed at 30 s, 1 min, 2 min, 5 min, 10 min, and 1 h following application of chlorhexidine.

Data analysis

After all imaging had been performed, the bacterial counts were analysed. A region of interest (ROI) was placed around each drop of chlorhexidine on the plates. From this ROI the total photon count was determined. Photon counts at each time point obtained from the same ROIs were compared to the baseline photon counts, which eliminated the need to ensure a homogeneous bacterial distribution on the Mueller–Hinton plates. All data were analysed using two-way analysis of variance with repeated measures and the Tukey–Kramer adjustment for multiple comparisons using SAS statistical software (SAS Institute, Cary, NC, USA) with significance set at $P < 0.05$.

Results

After 2 min of contact time, groups 1 and 2 had similar reductions in bacterial load with 30% of the original bacteria remaining in each group ($P = 0.512$). Group 3 had a significantly higher bacterial load (33%) when compared to both groups 1 and 2 (1 vs 3, $P < 0.0001$; 2 vs 3, $P = 0.0002$). The bacterial load in all three groups continued to decrease out to the final time point (1 h) with group 1 having the least amount of bacterial load remaining, 9% ($P < 0.0001$), and group 3 with the highest bacterial load remaining, 19% ($P < 0.0001$) (Figure 2).

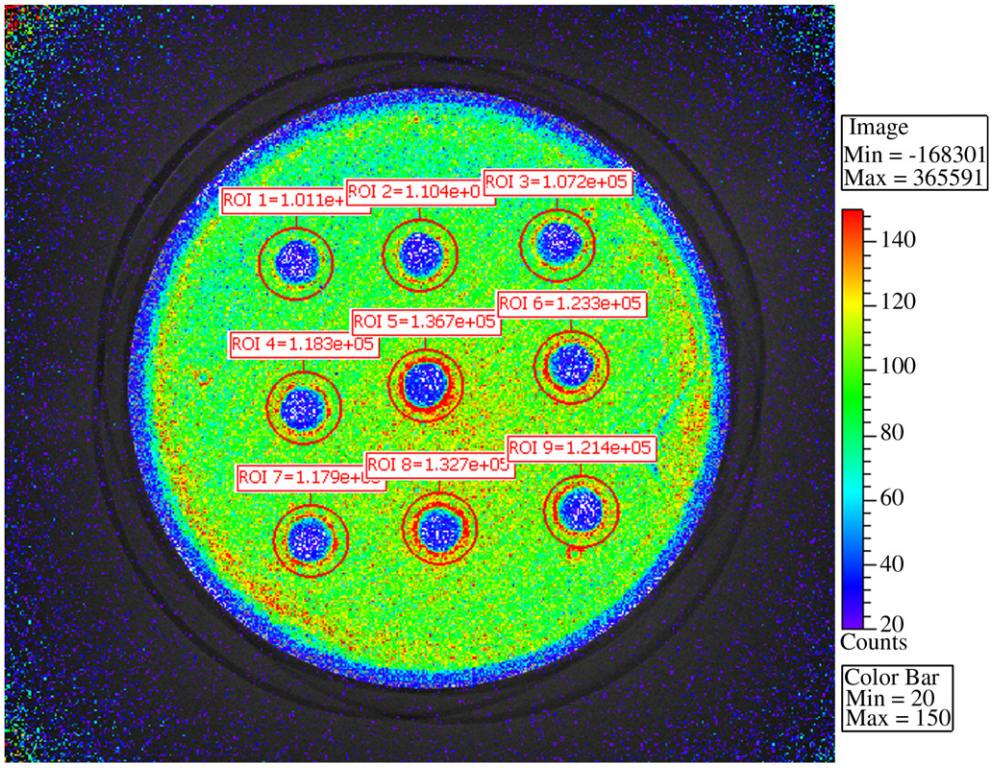

Figure 1. Representative luminescent image obtained following placement of nine drops of aqueous chlorhexidine. The region of interest (ROI) was selected and data collected from within each independent ROI during the entire time period.

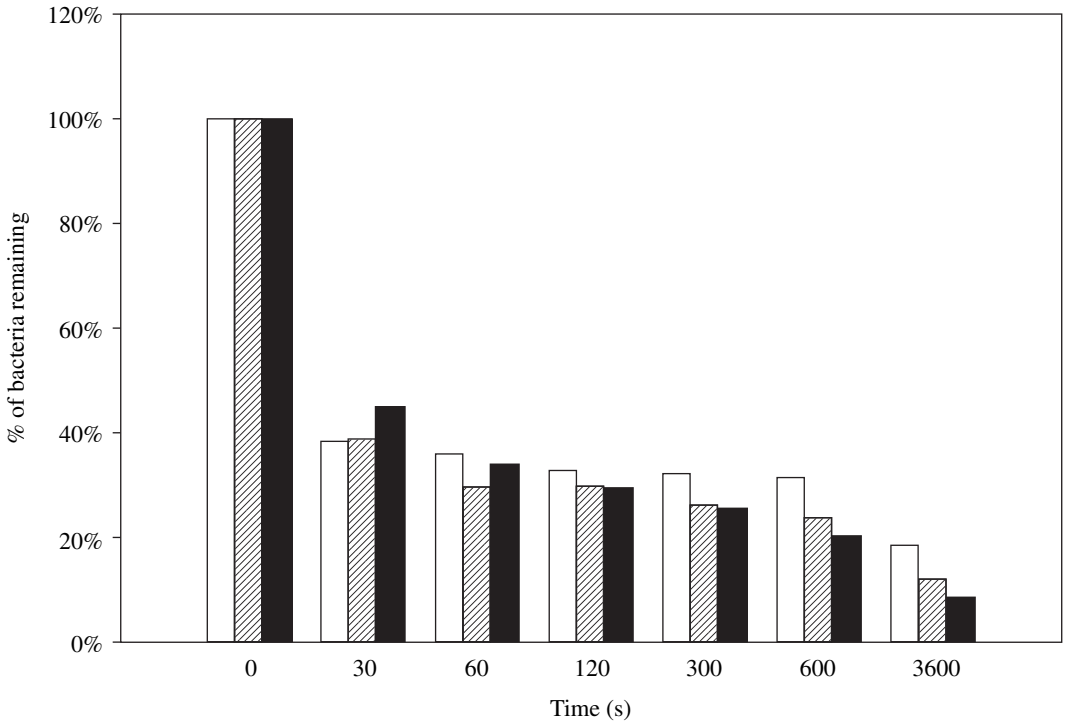

Figure 2. Bacterial load present after specified time intervals following treatment with chlorhexidine (white bars: 0.4%; hatched bars: 2.0%; black bars: 4.0%).

Discussion

All three groups demonstrated significant reductions in bacterial load after the first and second minute of contact time, with the higher concentrations of aqueous chlorhexidine resulting in greater reductions. Our results clearly demonstrate that chlorhexidine has a strong immediate effect, as well as a persistent effect, with continued reduction in bacterial load throughout the entire duration of the study.

Regarding skin preparation prior to insertion of central venous pressure catheters, the current guidelines from the Centers for Disease Control and Prevention recommend the use of chlorhexidine.[7] This is likely the result of multiple studies demonstrating reduced catheter site infections when chlorhexidine is used as a skin prep immediately prior to catheter insertion.[8] The superiority of chlorhexidine in skin antisepsis has also been shown when used prior to obtaining blood cultures. Caldeira *et al.* noted that the use of alcoholic chlorhexidine skin prep prior to venous puncture for blood cultures has resulted in a lower false-positive rate when compared to non-alcoholic povidone–iodine.[9] This is important to the treating physician, as a false-positive blood culture could result in unnecessary antibiotic treatment. The solution for the catheter-related infection is relatively simple: removal of the catheter. Unfortunately, the treatment of SSIs is not so easy, often requiring both antibiotics and surgical debridement. Despite this, the vast majority of the SSI literature has focused on only one surgeon-controlled variable, systemic antibiotic prophylaxis. Until recently, there has been little published information on preoperative skin antisepsis in the prevention of SSIs.

Bibbo *et al.* performed a prospective, randomized study comparing the effectiveness of chlorhexidine and povidone–iodine surgical skin preparation in clean, elective foot and ankle surgery.[2] Their study had two important results. First, chlorhexidine was more effective than povidone–iodine in minimizing positive bacterial cultures after the completion of surgical skin preparation. Although chlorhexidine demonstrated superior performance, 38% of patients still had positive cultures following the surgical skin prep. No time was given following surgical skin preparation regarding the aquistition of cultures, but they were all performed prior to skin incision and following a 7 min surgical skin prep. High rates of post-skin prep positive cultures were also seen in a study by Saltzman *et al.*, evaluating different skin prep solutions used in clean orthopaedic shoulder surgery.[10] Overall rates of positive cultures following skin prep were as high as 31% in the group receiving a povidone–iodine skin prep. ChloraPrep performed the best, and positive cultures of the post-skin prep operative site were obtained in 7% of patients. Similarly to the study by Bibbo *et al.*, no description was given regarding when the cultures were taken in relation to completion of the skin prep. These two studies demonstrate two important factors: first, that the skin prep does not eliminate all bacteria; second (not mentioned within the methods of either study), surgeons do not emphasize specific contact time when performing preoperative skin antisepsis. Our data suggest that contact time is important and could potentially further reduce the risk of SSIs due to persistent antimicrobial effect.

More recently, Darouiche *et al.* performed a multi-centre, prospective, randomized clinical trial comparing the number and types of SSIs in patients receiving preoperative surgical prep with a single application of 2% chlorhexidine gluconate and 70% isopropyl alcohol (ChloraPrep, Cardinal Health) or 10% povidone–iodine (Scrub Care Skin Prep Tray, Cardinal Health) scrub followed by paint.[1] Those treated with chlorhexidine–alcohol had significantly fewer overall SSIs when compared to povidone–iodine, 9.5% vs 16.1%. On further analysis based on type of SSI, there were significantly fewer superficial incisional and deep incisional infections. No difference was seen in organ-space infections. As demonstrated in this study, along with others, the majority of SSIs are confined to the skin; therefore, every effort taken to minimize the skin contamination prior to the surgical incision may further reduce the risk of SSIs.[1,11] Although the present study was unable to

show superiority with the higher concentration of chlorhexidine, the importance of contact time in minimizing bacterial contamination was demonstrated.

A meta-analysis by Noorani *et al*. demonstrated the superiority of chlorhexidine in minimizing SSIs when compared to povidone–iodine.[3] They combined data from six studies meeting their inclusion criteria, resulting in the overall analysis of 5031 patients. SSIs occurred in 6.1% of those who received chlorhexidine, compared to 9.8% of those who received povidone–iodine. Of note, the chlorhexidine concentration varied from 0.5% to 4% in the studies included for analysis, demonstrating the need for further research to optimize the concentration used for skin antisepsis.

This study has several limitations. First, we did not compare the time-dependent effect of aqueous chlorhexidine to other surgical skin preparation solutions, as the purpose of this study was to solely evaluate the time-dependent effect of aqueous chlorhexidine at various concentrations. Chlorhexidine alone was chosen for evaluation due to the growing body of literature demonstrating its superiority in skin antisepsis. Povidine–iodine, another commonly used skin preparation solution, was not tested because its mechanism of action, which requires drying in order to kill bacteria, would not allow for a fair comparison with chlorhexidine in the manner tested. Mueller–Hinton plates were chosen for this study to allow a large, evenly distributed number of bacteria with which the material tested could interact. Whereas Mueller–Hinton plates do not directly replicate the clinical setting, they serve as a 'worst case scenario' to allow direct evaluation of the effect of chlorhexidine on *S. aureus* without confounding variables that might be present in an *in vivo* model. Finally, only a small amount of chlorhexidine was used at the various concentrations. This was done because it would have been more difficult to determine the time-dependent effect of chlorhexidine if a larger amount of chlorhexidine had been used, resulting in a rapid, high bacterial kill.

As described previously, there is mounting evidence demonstrating the superiority of chlorhexidine in preoperative skin antisepsis, especially when used in conjunction with an isopropyl alcohol solution. As a result, many surgeons are using alcoholic chlorhexidine preps, whether in the form of a single application, i.e. ChloraPrep, or a standard chlorhexidine scrub followed by an isopropyl alcohol paint. Alcoholic chlorhexidine preps are appealing due to their ease of application as they are commercially available in a single use applicator, but some surgeons prefer non-alcohol-based preps due to the volatility associated with alcohol and risk of surgical fires that have been reported in the literature.[12,13] Due to alcohol's rapidity of action on cell death, it was not used within this study as the purpose of the study was to evaluate the time-dependent effect of aqueous chlorhexidine at different concentrations. Despite this, the addition of alcohol would likely result in further, more rapid, bacterial reductions.

In conclusion, while standard aseptic technique should always be adhered to, we recommend recording the period of time from prep completion to the time of skin incision to ensure adherence to manufacturer guidelines in an effort to minimize SSIs. We also recommend refraining from wiping the proposed surgical incision site dry of antiseptic solution prior to making the surgical incision, as the present study demonstrated the persistent antibacterial effect of chlorhexidine with contact time.

Conflict of interest statement

None declared. The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or US Government.

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